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GENERAL REMARKS ON THE TECHNIQUE OF TESTING FUNGICIDES 'IN VITRO' *

Up to ten or fifteen years ago, the methods of testing fungicides were entirely empirical. They were based on experiments in the open field, more or less amply repeated under different natural conditions, and the final results were interpreted by making a synthetic or gross estimate which reflected more the opinion of the observers than objective testing. If, after a sufficient number of years of experiments in divers environments, the fungicide produced the desired effect, the product was considered as approved.

The difficulties which beset this empirical method of testing are extremely numerous and are all the greater and more serious the more limited the number of experiments, the shorter their duration, or the less varied the conditions under which they are carried out.

A classic example of these difficulties is found in the case of Bordeaux mixture, which was not employed in practice until two years after the observation made by MILLARDET in October, 1882, because during the years 1883 and 1884, infection of vines by the downy mildew was practically nil, and it was only in 1885 that attack by this parasite enabled the efficacy of the lime copper wash to be proved.

The difficulties opposed to an exact valuation of the fungicidal effect of a product tested in the open field, are, *inter alia*, the following :–

(1) Practical evaluation is only clear and evident in the two extreme cases in which the fungicide either shows no efficacy at all or else is fully effective. In all the intermediary cases in which a certain action is observed, sufficient data are not available to calculate the actual degree of efficacy.

(2) The efficacy of a fungicide is the result of different characteristies which influence each other, by increasing or diminishing protection egainst the parasites. Since the method of testing in the open field only leads to a final general estimate of the degree of efficacy, if the latter is not complete, there is no way of improving the product or of attenuating its defects and increasing its favourable qualities.

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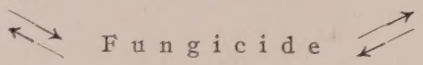
* Communication from Prof. RAFFAELE CIFERRI, Director of the Fungicidal Research Centre attached to the Botanical Institute and Cryptogamic Laboratory, Pavia, Italy.

Complete data on the subject treated in this communication will be published in the 'Atti' of the Botanical Institute and Cryptogamic Laboratory of Pavia.

(3) The efficacy of a fungicide is also the result of its action on both the fungus and on the host plant. It is therefore, all things considered, the result of a complex ternary, biological equilibrium :—



This delicate equilibrium, in general, is not analyzable in the aggregate results observed in the open field and which represent the sum of the negative and positive interactions. It is particularly difficult to evaluate the injurious action (especially the toxic) and the oligotropic action of a fungicide on the host plant, likewise the reactions which ensue on the inter-relations of the biological host + parasite couple, and which may lead to an increase or to an attenuation in defence against the parasite.

(4) The variables which influence the evaluation of a fungicide tried out in the open field are numerous and little studied ; they all have a tendency to dis tort the verification of the efficacy of a fungicide, either over- or under-estimating it.

These basic variables are :

(A) Relating to the host plant :–

(*a*) Intrinsic susceptibility of the plant (species or strain) to the parasite ;

(*b*) Vigour of the plant consonant in its turn with constituent elements, with cultural and agronomic factors in general, with age, the effect of previous diseases or disorders, etc.) ;

(*c*) Co-existence of other parasites, diseases or disorders ;

(*d*) Variability of micro-ambient factors (micro-edaphic, micro climatic, exposure and position of terrain, proximity of other plants, etc.).

(B) Relating to the parasite :–

(*a*) Inoculated mass of the parasite ;

(*b*) Virulence of the parasite, also in regard to the presence of eventual biological forms or races ;

(*c*) Totality of transport and rate of diffusion of the parasite, etc.

(C) Relating to the disharmonic host plant parasite symbiosis :—

(*a*) Conditions regarding contact between the host and the parasite ;

(*b*) Conditions regarding the establishment of infection (acquiring of infection) ;

(*c*) Uniformity of treatments, from the standpoint of both unit quantity and distribution of the fungicide on the plants ;

(*d*) Addition of different previous or successive treatments, etc.

Against these too numerous and, by their nature, too varied variables, the means of evaluating fungicidal efficacy amount to very little :–

(*a*) Yield in tons of product ;

(*b*) Some of its organoleptic characteristics and its chemical composition ;

(*c*) Appearance of certain pathological facts (for example, defoliation, malformations, atrophy, etc.) ;

(*d*) Unusual appearance of normal physiological phenomena outside the season (for instance, the appearance of anthocyan, defoliation, etc.).

All elements which, in general, are all the less evaluable in that the exact relation between the diseases and these effects are usually not known. For example, a particular which could form the basis of the evaluation of the damage caused by a parasite (and consequently the efficacy of fungicidal protection), and which however, is practically never taken into account, is the unit increase in dry weight of the parasitized plant in comparison with the non-infected control plants.

It should be added also that in testing the efficacy of a fungicide, two controls are necessary : the plant fully protected against the parasite and the plant completely unprotected. This implies double work and likewise twice the number of unknown factors.

The enumeration of all the difficulties encountered in the case of determining the true value of a fungicide, justifies, in our opinion, the establishment and development of a laboratory research technique on the fungicidal capacity of a compound. This technique already developed in different countries assumes two diverse aspects:

(1) Testing 'in vitro', under completely artifical but schematized conditions, easy to reproduce and always identical, which give a conventional but uniform basis. This is a relative method of evaluation because it is comparative, which always refers to a standard fungicide compared with the products being tested.

(2) Testing 'in vivo' under conditions approaching the optimum natural conditions for the development of the disease and which can be reproduced to more or less the same degree — in which the uniformity is less, but in which the basis is not conventional. Here it is an absolute method of evaluation, in the sense that it expresses the effective protective capacity of the fungicide, even independently of the standard provided by the standard fungicide.

Despite the evident need for working out a well studied and uniform technique for testing fungicides, the first diffident attempts which date from 1910-11 were not, so to speak, followed up and it was only in 1930 that they were renewed in earnest.

An analysis of the qualities required of fungicides may be simplified as follows :

(1) Intrinsic toxicity of the preparation under ideal conditions.

(2) Toxicity of the preparation under the conditions which occur in nature and which is usually equal to the availability of the product or its active principle.

(3) Susceptibility of the fungus to the preparation.

These three factors should determine the three series of determinations to be effected in order to establish the fungicidal power of a preparation, namely :–

I. Determination of the toxicity of the preparation tested and under the conditions most favourable to its action which can be realized in the laboratory ;

II. Determination of the fungicidal power of the preparation under normal average conditions of infection in nature, even if reproduced artificially for the requirements of study ;

III. Determination, under known and controlled conditions, of the susceptibility of the fungus, which has to be combated, to the active principle of the preparation under study.

In their turn, however, the conditions (1), (2) and (3) depend upon numerous other factors, to a large extent unknown or barely imagined, and which, in the present state of knowledge, it is impossible to analyze separately.

Thus, the intrinsic toxicity of the preparation is related to : —

(*a*) The chemical nature and state of the fungicidal principle ;

(*b*) The chemical nature and state of the solvent ;

(*c*) The chemical and physico-chemical relation between the solvent and the solution ;

(*d*) The effect produced by the substances added to the solvent and to the solution ;

(*e*) Manner of preparation of the fungicide.

The toxicity of the preparation under practical conditions depends on an ensemble of still more complex conditions which may be assembled into :

(*a*) Initial retention (or initial fungicidal capacity) of activity, that is to say, the quantity of the active preparation which remains adherent to the treated parts of the plant, immediately after spraying ;

(*b*) Residual retention (or residual fungicidal capacity) of activity, namely, the quantity of preparation which remains adherent to the treated parts, after having undergone deterioration through weather agents, known as 'tenacity' by English investigators.

This involves two orders of determinations which are : —

IV. Testing of the fungicidal power of the preparation after spraying of inoculated plants before the product deteriorates through the action of weather agents ;

V. Testing of the fungicidal power of the preparation after it has deteriorated through weather agents, either in the open under natural conditions, or under weather conditions artificially reproduced in the laboratory.

In short, the complete study of a fungicide comprises the following successive investigations :—

I. Determination of the fungicidal power 'in vitro' in the laboratory under optimum conditions of action ;

II. Determination as above, but under conditions of less efficacy (generally with different and successive dilutions) in order to determine the limits of efficacy of the preparation ;

III. Determination of the fungicidal power 'in vivo' under glass or in the open, without the preparation having undergone the deteriorating effect of weather or other agents ;

IV. Determination as above after the preparation has undergone deterioration through weather or other agents ;

V. Determination of the susceptibility of the fungus, which has to be combated, to the active principle of the fungicide, in order to distinguish its action from the depressing or stimulating action of other substances present in the preparation, as well as the influence of mode of preparation.

CROP PESTS OBSERVED IN RUMANIA *

Leaving aside the universally widespread crop pests observed every year, I will limit this note to the species newly introduced into the country and to those which, while being endemic and distributed over a considerable part of the country have attacked and damaged vegetal species other than their customary host-plants and which consequently merit special attention.

Among the plant pests which made their appearance in Rumania during the 1942 summer, mention should be made in the first place of the pea aphis (*Acyrthosiphon onobrychis* Boyer). Formerly, this parasite was observed every year forming colonies on leguminous plants cultivated in gardens and in the fields, but these colonies were more or less isolated and never attained a number of individuals and an extension susceptible of causing annoyance or serious damage. The drought of the 1942 summer favoured the breeding of this aphis, with the result that there soon occurred in certain districts of the country, Moldavia and Oltenia in particular, a mass development, never before observed, which caused the destruction of important pea and vetch crops.

The European corn borer (*Pyrausta nubilalis* Hübner) this year attacked some crops of hemp. Their invasion in the Danube Valley by this moth generally harmful only in places, was favoured by the want of maize crops in the localities in question owing to the unfavourable climatic conditions during the autumn and spring which hindered agricultural operations. The losses, in some places, amounted to 20 per cent., though only on small areas. On harvesting the hemp, the caterpillars were in the final phase of their development and part had already turned into pupas.

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* Communication from the official correspondent of the International Institute of Agriculture, Prof. Dr WILHELM KNECHTEL, Chief of the Entomological Section, Institute of Agricultural Research of Rumania, Bucharest, Rumania.

Thrips lini Ladureau (*Th. linarius* Uzel) was observed for the first time during the 1942 summer on flax crops in the vicinity of Bucharest. It was not known until now whether this insect, commonly called the 'flax fly' and exerting when present in large numbers, an injurious influence on the growth of this plant, formed part of the fauna of Rumanian Thysanoptera. *Th. lini*, therefore, should be considered as new to the country, a fact interesting not only from the viewpoint of fauna, but also from that of Rumanian agriculture as the flax crop is the object of particular attention and is being grown on increasing areas. It would be useful to amplify observations and researches. Up to date, the new parasite has only been observed in the form of isolated individuals attached to the tips of the flax shoots and not causing any appreciable damage. Account should be taken, however, of the possibility of a dangerous development in mass, if conditions favourable to their multiplication were to arise.

As other harmful Thysanoptera, mention could be made of: *Heliothrips femoralis* Reuter, *Taeniothrips dianthi* Priesner and *Thrips tabaci* Lindemann.

Hel. femoralis was first observed in a greenhouse on the under surface of a leaf of *Cineraria* amidst a colony of aphids composed of *Macrosiphon koehleri* Börner, *Myzus ornatus* Laing and *Myzodes persicae* Sulzer. Several individuals were subsequently discovered on azaleas cultivated in another greenhouse. This species, likewise *Hel. haemorrhoidalis* Bouché which is observed fairly frequently, but solely on greenhouse plants, only attacks plants cultivated in closed warm premises. It does not seem to be very widespread in the country where it was probably introduced only recently through the importation of greenhouse plants.

Taen. dianthi, another species new to Rumania, for the first time since two years, invaded a carnation crop in the region of Bucharest causing damage. The attack of this parasite caused dwarfing of the plants and shrub development without the formation of buds. The leaves were greatly deformed. The few flowers were spotted and of no value. The carnation crop was completely destroyed; this parasite also seems to have been introduced through importation of carnations from countries of Southern Europe.

The carnation crops suffered damage of the same kind though to a smaller extent through *Th. tabaci*: a species widespread throughout Rumania and attacking different species of wild and cultivated plants.

Greenhouse and house plants were, in nearly all cases, attacked by *Macr. koehleri*, *Myz. ornatus*, *Myz. persicae* and *Neomyzus circumflexus* Buckton. It should be noted that *Myz. persicae* is found fairly frequently and on different greenhouse plants, while it is rather unusual on peach-trees in the open. In this case *Appelia schwartzi* Börner is to be found most often.

Pteronus ribesii Scopoli caused considerable damage to red current crops near Sibiu in Transylvania. The destruction of the leaves resulted in an appreciable reduction in yield.

The onion fly (*Hylemyia antiqua* Meigen) destroyed small onions newly planted over more or less extensive areas in the gardens of Ploești.

In the summer *Tylenchus dipsaci* Kühn was observed to have attacked onions having practically attained full development.

Among the mites, *Tetranychus althaeae* v. Hanst. appeared in the wine-growing region of Odobești and favoured by the pronounced summer drought increased by mass breeding of an epidemic character. This phenomenon was probably considerably accentuated by the want of sulphuring the vines for the control of vine mildew (*Uncinula necator*). The varieties ' Traminer ', ' Aligote ' and ' Cabernet ' suffered considerable damage.

There remains to be mentioned the observations made on the influence, exercized by the exceptionally severe and long winter of 1941-42, on the development of two scale insects. It was found, in fact, in the spring that the nurseries invaded by the San José scale (*Aonidiella perniciosa* Comstock) that practically 100 per cent. of the insects had been destroyed. With brown apricot scale (*Lecanium corni* Bouché), the mortality was as much as 50 per cent.

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* Drawn up by Giulio Trinchieri, International Institute of Agriculture.

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